



### Inside this issue:

Comparison of Permeabilization Buffers to Evaluate Intracellular Protein Expression. 1

Upcoming Events 1

FCS Laboratory Fourth Quarter 2013 Updates 1

Winter Fun in Washington 1

### Upcoming Events:

- ◆ Society of Toxicology Annual Meeting, Phoenix, AZ, March 2014
- ◆ AAPS National Biotech Conference, San Diego, CA, May 2014
- ◆ ISAC, Lauderdale, FL., May 2014



## Comparison of Permeabilization Buffers to Evaluate Intracellular Protein Expression

By: Lynette Brown, MS, CHT and Jennifer J Stewart, PhD

The introduction of peptides, such as antibodies, into mammalian cells has been an invaluable biochemical approach in the study of intracellular processes, especially in the staining of cytoplasmic antigens for flow cytometry enumeration. FoxP3 is a cytoplasmic transcription factor expressed in T regulatory cells and has been widely used in the identification of these cells.

In this article, we have highlighted the main points of our comparison of five different fixation and cell permeabilization methods (listed below) and the percent positive T regulatory cells (T Regs) defined as CD45+CD3+CD4+FoxP3+CD25+CD127+ by surface and intracellular flow cytometry.

**\*Buffer 1**-BD Pharmingen FoxP3 Buffer Set

**\*Buffer 2**-BD Pharmingen Transcription Factor Buffer Set

**\*Buffer 3**-Proprietary FCSL Intracellular Buffer Set

**\*Buffer 4**-Method published in Chow et al., 2005

**\*Buffer 5**-Biolegend FoxP3 Fix/Perm Buffer Set

From the table, it is observed that there is a large variability between buffer set condi-

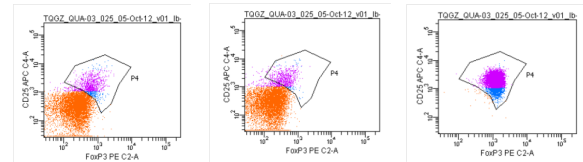
### FCS Laboratory Fourth Quarter 2013 Updates

\*Last quarter, in lieu of producing a newsletter, we focused our efforts on the Chase Mission Main Street Grant Program. Many thanks to all who helped us qualify by voting for us with your Facebook accounts! Winners will be announced in January, 2014.

Fix and Permeabilization Buffer Comparison

Human ID	Marker	Buffer 1	Buffer 2	Buffer 3	Buffer 4	Buffer 5	Mean	SD	%CV
01		7.0	6.2	9.1	3.8	85.6	22.34	35.41	158.52
02	T Regs	8.3	7.7	10.3	3.2	85.0	22.90	34.81	152.02
03		8.4	7.6	10.1	3.9	84.8	22.96	34.64	150.89

tions. Evaluation of CD45+ staining for all the buffer sets, Buffer 3 and 4 both showed an observable decrease in CD45 signal intensity (data not shown).



From left to right, Buffer 1, 2 and 5 are shown as FoxP3 vs. CD25 staining. From these data we have shown that Buffer 1 gave the best distinction in the cell population, but Buffer 2 is a good alternative.

If you would like more detailed information about this comparison, please contact us!

### Winter Fun in Washington

\* The Seattle Boat show (Jan 24th—Feb 2nd) is the West Coast's largest boat show featuring 1000's of watercraft at Century Field and South Lake Union. Visit [www.seattleboatshow.com/](http://www.seattleboatshow.com/) for details.



13029 NE 126th PL  
Kirkland, WA 98034  
Phone: 425-821-3900  
Fax: 425-821-3925  
Email: [info@fcslaboratory.com](mailto:info@fcslaboratory.com)



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